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Advances in the valorization of spent brewer's yeast

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ABSTRACT

Spent brewer's yeast (SBY) is one of the major by-products produced during beer brewing process. SBY is an abundant source of protein, minerals, vitamins, especially vitamin Bcomplex, as well as nutraceuticals such as β -glucans or mono- and oligosaccharides. Due to the presence of nutrients, abundant availability and low cost, SBY has been widely used in animal feed. However, over the last decades, considerable efforts have been devoted to the development of alternative applications for SBY, such as functional food ingredient and fermentation substrate. Therefore, the aim of this review was to provide an up-to-date overview on the valorization of SBY.

1. Introduction

Conventionally, brewer's yeast strains are divided into two categories, namely top-fermenting (ale) and bottom-fermenting (lager) yeasts. Strains of *Saccharomyces cerevisiae* are commonly used to produce ale beers in the temperature range of 16–25 °C. On the other hand, *Saccharomyces pastorianus* or *Saccharomyces carlsbergensis* strains are industrially used to produce lager beers in the temperature range of 8–15 °C (Ferreira, Pinho, Vieira, & Tavarela, 2010).

In beer brewing process, spent brewer's yeast (SBY) (also known as residual yeast or surplus yeast) is one of the predominant by-products. Other major by-products include brewery spent grain and hot trub. SBY accounts for approximately 1.5–2.5% of the total beer production (Bekatorou, Plessas, & Mantzourani, 2015). In a lager fermentation, total amount of SBY is approximately 0.6–0.8 lb./bbl (pounds per barrel) of final volume of product (Huige, 2006). SBY is an abundant and inexpensive source of protein (45–60%), minerals, Bcomplex vitamins and other worth constituents (Podpora, Świderski, Sadowska, Rakowska, & Wasiak-Zys, 2016). In addition, spent yeasts are a rich source of nutraceuticals such as β -glucans or mono- and oligosaccharides.

SBY is generally regarded as safe (GRAS) for human consumption (Ferreira et al., 2010). However, due to high levels (6–15%) of nucleic acids in their composition, SBY application in human nutrition as a protein source is limited (Podpora et al., 2016). It is well-known that high nucleic acid levels in the human diet may lead to increased blood uric acid levels and in turn can result in hyperuricemia (Podpora et al., 2016). Therefore, SBY has been conventionally used as a cheap source of protein in animal diets. Because of certain disadvantages with SBY

such as low shelf life, transportation cost and requirement for further processing (Boateng, Okai, Frimpong, & Zeebone, 2015), spent yeasts are usually disposed off in the environment. Nevertheless, the valorization of SBY can be achieved through the extraction and isolation of economically important components such as proteins, amino acids, bioactives like β -glucans and functional peptides, vitamins, minerals, fiber, flavor compounds and others.

2. SBY composition

SBY contains relatively high levels of protein and low levels of reducing sugars; and total organic carbon content was estimated to be approximately 45% dry matter (DM) (Mathias, Alexandre, Cammarota, de Mello, & Sérvulo, 2015). Approximate levels (% DM) of ash content, total nitrogen and total protein in SBY were shown to be 6, 9 and 45.6, respectively; and average levels (% DM) of soluble reducing sugar, soluble nitrogen and soluble protein were estimated to be 1.3, 2.53 and 14.66, respectively (Mathias et al., 2015). In addition, the average levels of free amino nitrogen and chemical oxygen demand were calculated to be 4.09 mg/g and 1308 mg/g, respectively (Mathias et al., 2015).

3. Yeast cell disruption

Different mechanical and non-mechanical techniques can be used for yeast cell disruption. Milling and homogenization techniques fall under mechanical category. Non-mechanical techniques can be further divided into physical, chemical, enzymatic and electrical, as shown in Fig. 1.

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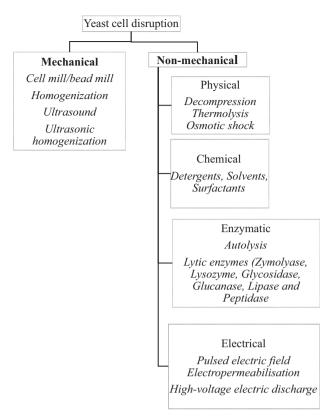


Fig. 1. Different methods for the disruption of yeast cell. (Adapted with permission from Liu, Ding, Sun, Boussetta, and Vorobiev (2016).)

4. Potential valorization opportunities for SBY

4.1. Rich source of β -glucans

β-D-Glucans possess several physiological functions. Spent brewer's yeast (SBY) cell walls are rich in β-D-glucan. Breweries could generate additional revenue by isolating β-glucan from SBY as a high-value product. Tian, Yang, and Jiang (2019) used alkali treatment at high pressure for the isolation of β-D-glucan from SBY. They showed that, at optimal conditions, extraction rate of 78.38% with β-D-glucan content of 78.11% can be achieved. Homogenization of cell walls was found to yield higher β-glucan content (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). Alkaline treatment was used to isolate particulate (water-insoluble) β -glucan from SBY cell walls and β -glucan with minimal structural changes can be obtained through the combination of sonication with spray-drying, and the formed particles exhibited insignificant agglomerates formation (Zechner-Krpan et al., 2010). For most food applications, β -glucan powders with free-flowing properties are desirable. β-Glucans can improve the functional properties of food products by acting as thickening, emulsifying, oil-binding or water-holding agents (Thammakiti et al., 2004). However, these capacities of B-glucans were shown to be affected by differences in isolation and drying procedures (Petravić-Tominac et al., 2011). They found that lyophilized preparations exhibited highest oil-binding capacity and lowest swelling and air-dried preparations showed enhanced swelling. It has also been shown that β-glucans obtained from homogenized cell walls of SBY exhibited relatively higher apparent viscosity, emulsion stabilizing capacity and water-holding capacity compared with commercial β-glucan from baker's yeast (Thammakiti et al., 2004).

 β -Glucans obtained from SBY have been used in yoghurts as nutraceutical ingredient—addition up to 0.3% did not affect the structure stability and sensory quality of natural yoghurts (Piotrowska,

Waszkiewicz-Robak, & Swiderski, 2009). Powdered β-glucan derived from SBY was used as a thickener in skimmed-milk yogurt (Raikos, Grant, Hayes, & Ranawana, 2018). They showed that the powder incorporation did not negatively affect most of the physicochemical properties of the yogurt. β-Glucans derived from SBY were used as functional ingredient in bread (Martins, Pinho, & Ferreira, 2018). They showed that β -glucan-rich extract contributed to improved β -glucan and dietary fiber contents in bread. In addition, β -glucans incorporated bread exhibited higher specific volume with uniform pores. Furthermore, β-glucans promoted bread crust browning. The potential of βglucan derived from SBY as a fat replacer in mayonnaise has been shown (Marinescu, Stoicescu, & Patrascu, 2011; Worrasinchai, Suphantharika, Piniai, & Jamnong, 2006). Marinescu et al. (2011) showed that storage stability of mayonnaise was improved with β glucan substitution at a level of 50%. Their results concluded that SBY β-glucans can be used as emulsion stabilizer and fat replacer in mayonnaise. However, β-glucan incorporation may adversely affect sensory quality of reduced-fat mayonnaise, especially appearance and color. As a remedy to this drawback, the application of natural colorants (e.g., \beta-carotene and lutein) has been recommended (Santipanichwong & Suphantharika, 2007). The addition of β-glucan from SBY to starch-based food (up to 1%) was suggested to be advantageous to reduce calorie content as well as to maintain desirable textural characteristics (Yoo & Lee, 2007). β-Glucan prepared from SBY was successfully used to retard gel hardness development of rice starch/ β-glucan mixed gels during long-term refrigerated storage (Satrapai & Suphantharika, 2007). Fine particulate β-glucans from SBY have been shown to contribute to strengthening of gel network formed by SBY βglucans and κ -carrageenan (Xu et al., 2009). Their findings could be useful for developing novel foods. Applications of yeast-derived β-glucans in food production have been reviewed by Zechner-Krpan, Petravić-Tominac, Panikota-Krbavčić, Grba, and Berković (2009).

β-Glucan-rich fraction prepared from SBY has been used as an immunostimulant in shrimp feed (Thanardkit, Khunrae, Suphantharika, & Verduyn, 2002). They showed that autolysis and further treatment with hot alkali of SBY led to the production of a fraction containing an apparent glucan content of approx. 53% (w/w). Upon short-term (3 days) feeding this impure β -glucan to shrimp at 0.2% (w/w of the feed), considerable increases in the number of haemocytes, phenol oxidase and bactericidal activity (against Vibrio harveyi) were observed. In another study by Suphantharika, Khunrae, Thanardkit, and Verduyn (2003), β-glucan-rich fraction originated from the insoluble cell wall fraction of SBY was shown to exert an increased phenoloxidase activity in hemolymph of black tiger shrimp under both in vitro and in vivo conditions when compared with controls. SBY β -D-glucan was used as a biological response modifier (BRM) (Liepins et al., 2015). They showed that immunogenic properties (TNF-α induction activity) of SBY β-Dglucans were improved by desiccation; furthermore, dried SBY carboxymethylated β-D-glucan was shown to induce immunoactivity similar to or exceeded that of pleuran (β-glucan from *Pleurotus ostreatus*), a well-characterized fungal BRM. β-Glucan extracted from SBY can potentially be used as a cryoprotectant for probiotic Lactobacillus cultures during freeze drying, storage (4 °C) and in vitro digestion (da Silva Guedes et al., 2019). B-Glucans isolated from SBY were found to be more effective at lowering the levels of LDL cholesterol and triacylglycerols as well as serum total cholesterol in experimental rats (Waszkiewicz-Robak & Bartnikowska, 2009). B-Glucans derived from SBY could be used for the adsorption of mycotoxins since β-D-glucans isolated from yeast cell walls have been shown to exhibit a good adsorption capacity for zearalenone (alkali-insoluble fraction: up to 50%; alkali-soluble fraction: ~16%) (Yiannikouris et al., 2004).

4.2. Source of proteins and functional peptides

SBY extracts are rich in proteins (Vieira, Cunha, & Ferreira, 2019). Yeast cell disruption is a crucial step for maximum protein recovery.

The potential of subcritical water to hydrolyze SBY cells and subsequent recovery of proteins and amino acids has been shown (Lamoolphak et al., 2006). They showed that, with an increase in temperature of the water, the yield of protein increased and the yield of amino acids decreased; the maximum amino acid and protein yields obtained were 0.063 and 0.16 mg/mg of dry yeast, respectively. Marson, da Costa Machado, de Castro, and Hubinger (2019) compared conventional approaches (autolysis and mechanical rupture) with enzymatic hydrolysis for the isolation of proteins. Their results showed that sequential enzymatic hydrolysis (with Brauzyn® and Alcalase™) resulted in maximum protein yield and antioxidant properties. Amorim et al. (2016) obtained peptide concentrates from hydrolyzed SBY proteins via filtration. They showed that peptide extracts, especially low molecular weight peptide fraction (< 3 kDa), exhibited significant antiulcer activity. In addition, they showed the antiproliferative activity of the extracts against leukemia cells. In another study, Amorim, Pinheiro, and Pintado (2019) have optimized SBY autolysis and subsequent hydrolysis processes by response surface methodology for the generation of stable angiotensin converting enzyme (ACE)-inhibitory peptides. They found that a peptide fraction (< 3 kDa) exhibited potent ACEinhibitory activity and superior in vitro gastrointestinal stability. Their results indicated that SBY can be used as a source of ACE-inhibitory peptides, which are well-known to exhibit antihypertensive effect. For effective separation of polysaccharide and protein from SBY extract, the use of ultrafiltration technology has been recommended (Huang, Gao, Ma, & Lu, 2012).

4.3. Autolysis and functional quality

Enzymatic treatment of SBY during autolysis has been shown to improve functional quality of extracts derived from SBY. Cao et al. (2017) showed that pretreatment with β -mannanase during SBY autolysis had substantially enhanced the yield of SBY extracts through improved cell wall degradation (polysaccharides were converted into oligosaccharides). In addition, enhanced antioxidant activity was noted in the enzyme-pretreated extracts. Autolysis of SBY extract (from inner cell content) was optimized by response surface methodology in order to obtain SBY autolysate possessing enhanced ACE-inhibitory and antioxidant properties (Vieira, Melo, & Ferreira, 2017). Efficient *in vitro* absorption of bioactive compounds from SBY autolysate has been shown (Vieira et al., 2016). A two-step autolysis has been shown to be result in improved amino acid yield from SBY (Boonyeun, Shotipruk, Prommuak, Suphantharika, & Muangnapoh, 2011).

4.4. Functional food ingredient

SBY extracts have been shown to be a promising source of bioactive ingredients, since they contain well-balanced amino acid profile along with other functional constituents such as phenolic acids, flavonoids, carotenoids and peptides (Vieira et al., 2019). SBY autolysate was supplemented to improve the nutritive value of beetroot and carrot juices (Rakin, Vukasinovic, Siler-Marinkovic, & Maksimovic, 2007). They showed that the autolysate contributed for improved lactic acid production and the fermented juices possessed enhanced levels of minerals, pigments and vitamins. The incorporation of SBY extract (1%) as additive has shown to improve free amino acid and protein contents, chewiness and hardness (acts as gel stabilizer) of cooked ham (Pancrazio et al., 2016). Yeast extracts rich in essential amino acids, low molecular weight peptides, and with high antioxidant activities were prepared from SBY (Podpora et al., 2016). They suggested that these extracts could be used for the design of dietary supplements and functional foods. SBY hydrolysate rich in Cyclo-His-Pro (CHP) content was prepared by enzymatic hydrolysis and the hydrolysate showed significant antioxidant and/or antidiabetic properties (Jung et al., 2011), and therefore it can potentially be used for the preparation of functional foods.

Yeast mannoproteins are approved food additives, especially as a stabilizer in wine. SBY has been shown to be a viable biomaterial for obtainment of extract rich in mannoproteins (Costa, Magnani, & Castro-Gomez, 2012). They showed that the extract exhibited good emulsification activity and emulsion stability. Mannoprotein derived from SBY has the potential to be used as an emulsifier/stabilizer in French salad dressing (de Melo, de Souza, da Silva Araujo, & Magnani, 2015). They showed that salad dressing prepared with mannoprotein derived from spent Saccharomyces uvarum exhibited the highest scores for color, flavor, taste and overall acceptance. In another study, β-glucan and mannoprotein were extracted from SBY cell wall with satisfactory purity and high yields (da Silva Araújo et al., 2014). They showed that the obtained mannoprotein possessed good stabilizing and emulsifying properties, and can potentially replace xanthan gum in mayonnaise formulations without negatively affecting their sensory properties during refrigerated storage.

SBY, especially from craft brewery, contains hop acids (iso α -, α -, and β -acids) at elevated levels (Bryant & Cohen, 2015). These acids are known to have antibacterial, antioxidant, anticancer, and anti-inflammatory activities, and therefore spent craft brewer's yeast could be used as a valuable functional food ingredient.

4.5. Yeast extract

For the production of yeast extracts, SBY has been shown to be a nutrient-rich and cost-effective starting material (Jacob, Striegel, Rychlik, Hutzler, & Methner, 2019a). Their results also showed that the composition of SBY influences the proportion of physiologically important ingredients of yeast extract. Yeast extract from SBY was produced by autolysis, which was induced through incubation at elevated temperatures for different durations (Tanguler & Erten, 2008). Their results indicated that yeast extract with considerable levels of protein and α -amino nitrogen can be obtained from autolysis at 50 °C for 24 h. In another study by Jacob, Striegel, Rychlik, Hutzler, & Methner (2019b), for superior quality yeast extract production, cell disruptions by cell mill and sonotrode methods have been shown to be effective alternatives to traditional autolytic method. They showed that yeast extract produced by the mechanical methods contained relatively high levels of protein, fat, trehalose, Bvitamins, especially biologically active 5-methyltetrahydrofolate. SBY extract has been shown to be rich in protein, low in fat and moisture contents; and a potential source of essential amino acids as well as flavor enhancing amino acids (glycine, alanine, glutamic acid and aspartic acid) (Vieira et al., 2016). In addition, the extracts were shown to contain macrominerals (K, Na, Ca, Mg) trace elements (Zn, Fe, Mn, Cu, Cr, Co, Mo, Se) and vitamins (B3, B6 and B9). Yeast extract prepared from SBY has been used for growth and sporulation of Bacillus thuringiensis subsp. kurstaki, a well-known biological control agent against lepidopterans (Saksinchai, Suphantharika, & Verduyn, 2001). They showed that Fe was a limiting nutrient in the extract for complete sporulation to occur. The nutritional composition of SBY extract is given in Table 1.

4.6. Fermentation substrate

SBY has considerable potential to be used as additive in fermentation media because of its low C/N ratio (Mathias et al., 2015). SBY was used as alternative growth medium for the production of proteolytic enzyme via lactic acid bacteria cultivation (Mathias, de Aguiar, de Almeida e Silva, de Mello, & Sérvulo, 2017). They showed that SBY was the most suitable substrate among other brewery wastes for extracellular enzyme production, and glucose supplementation exerted a positive effect on the enzyme production since residual yeast are low in fermentable sugars (Mathias et al., 2015). For improving fermentation efficiency in home brewing or other settings, Vegemite (food spread manufactured from SBY extract) or other yeast extract spreads have been suggested as potential sources of readily available and inexpensive

Table 1Mean values of nutritional composition of spent brewer's yeast (SBY) extract.

(Adapted with permission from Vieira, Carvalho, et al. (2016).)

Proximate composition	(g/100 g dw)
Moisture (%)	7.7
Protein	64.1
α-amino nitrogen	3.7
Carbohydrates	12.9
Fat	1.3
Ash	14.0
Ribonucleic acid (RNA)	4.0
Macrominerals	(mg/100 g dw)
Potassium (K)	9148
Sodium (Na)	1228
Magnesium (Mg)	273
Calcium (Ca)	27
Trace elements	(mg/100 g dw)
Chromium (Cr)	0.019
Copper (Cu)	0.364
Cobalt (Co)	0.03
Manganese (Mn)	0.564
Iron (Fe)	1.76
Selenium (Se)	0.030
Zinc (Zn)	11.9
Molybdenum (Mo)	0.003
Vitamins	(mg/100 g dw)
Nicotinic acid (B3)	77.2
Pyridoxine (B6)	55.1
Folic acid (B9)	3.01
Riboflavin (B2)	< 0.32
Cyanocobalamin (B12)	< 0.25

nutrients (Kerr & Schulz, 2016). SBY has been used as one of the fermentation nutrients for the production of ACE-inhibitory proteins from *Ganoderma lucidum* mycelia (Mohamad Ansor, Abdullah, & Aminudin, 2013). The potential of SBY extract for cost-effective fermentative production of succinate has been shown (Sawisit et al., 2012). They showed that a final yield of 68.73% can be achieved by using glucose or lactose medium supplemented with SBY extract at 5 g/L. Enzymatic hydrolysate of SBY as a nitrogen source has been shown to successfully replace yeast extract for succinic acid production in glucose-containing media (Jiang et al., 2010) and in corn fiber hydrolysate (Chen et al., 2011) by *Actinobacillus succinogenes* NJ113. In both the studies, vitamins supplementation further enhanced the succinic acid yield.

4.6.1. Ethanol fermentation

SBY was used as a nutrient adjunct in ethanol fermentation using corn mashes (Pietrzak & Kawa-Rygielska, 2013). They showed that, depending on the level of SBY addition (0.1 to 5.0% w/w), the rates of sugar consumption and ethanol production were accelerated; and 6.5 to 11% improvement in overall ethanol yield was noted by the supplementations. SBY was supplemented as a nutrient source in a very high gravity (VHG) ethanol fermentation using maize mashes (Kawa-Rygielska & Pietrzak, 2014). They showed that SBY and derived products (spray dried yeast and yeast extract) supplementation led to increased ethanol production rates, ethanol yield and decreased fermentation time. Yeast extract produced by SBY autolysis (A-YE) was used as a substitute for commercial yeast extract in VHG ethanol fermentation of cassava starch (Palasak, Sooksai, & Savarajara, 2019). They showed that an ethanol yield of 115.77 g/L can be obtained in 24 h by the supplementation of 5.23 g/L of A-YE. For ethanol production, SBY was used as a low-cost feedstock (as inoculum) along with two other agroindustrial wastes, namely soft-drink industry wastewater as carbon source and corn steep water as nitrogen source (Comelli, Seluy, Benzzo, & Isla, 2018). They showed a complete utilization of sugars in the wastewater in < 8 h at optimal conditions, with 0.45 gethanol/gsugar ethanol yields. Despite these merits, SBY supplementation may lead to high concentrations of maltodextrins (un-fermentable by yeast) in mashes, and therefore, limiting the addition or additional treatment has been suggested to alleviate the disadvantage (Kawa-Rygielska & Pietrzak, 2014).

4.6.2. Renewable energy

SBY has been shown to be a viable source for methane (biogas) production through anaerobic digestion and SBY source influenced the biogas composition (Sosa-Hernández, Parameswaran, Alemán-Nava, Torres, & Parra-Saldívar, 2016). In another study, specific methane production and solubilization rate constant for SBY in fed-batch stirred reactors (5 L) were reported to be 0.255 L methane per gram of COD and 0.659 d⁻¹, respectively (Vitanza, Cortesi, Gallo, Colussi, & De Arana-Sarabia, 2016). Because of its rapid solubilization, SBY could be used as an effective substrate for co-digestion purposes. SBY as a cosubstrate was successfully used for methane generation from swine manure via anaerobic digestion (Spajic et al., 2010). SBY along with spent grain have been used as substrates for methane production through anaerobic digestion (Oliveira, Alves, & Costa, 2018). They showed that the combination of the substrates was more promising than individual substrate digestion in terms of produced methane volume; and maximum methane generation and biodegradability can be achieved by the addition of crude glycerol as co-substrate to these substrates. In another study, bio-oil and granular activated carbon were produced using SBY and brewer's spent grain through pyrolysis and CO₂ activation (Gonçalves, Nakamura, Furtado, & Veit, 2017).

4.6.3. Lactic acid fermentation

SBY was used as an inexpensive nitrogen source in L-(+)-lactic acid fermentation using brewer's spent grain (BSG) hydrolysate (Pejin et al., 2019). They showed that free amino nitrogen (FAN) concentration of the medium was significantly increased by SBY addition. In addition, the yield of L-(+)-lactic acid was reported to be 89% with 50 g/L of SBY. Brewing by-products, namely brewer's spent grain and SBY, along with malting and oil industry by-products (malt rootlets and soy lecithin, respectively) have been used as substrates in L-(+)-lactic acid fermentation (Radosavljević et al., 2019; Radosavljević et al., 2020). They found that the combination of these by-products formed an inexpensive and appropriate medium for lactic acid fermentation.

4.7. Feed ingredient

4.7.1. Aqua feed

Dietary supplementation with SBY was shown to improve digestive capacities of white seabream and meagre fish species (Castro et al., 2013). They showed that, with 2% SBY diet, improved protease and amylase activities were observed in the intestine and the pyloric caeca of white seabream. In addition, enhanced lipase activity in the pyloric caeca was noted. On the other hand, enhanced amylase activity was noted in the pyloric caeca of meagre with the supplementation. The potential of SBY as a fish meal alternative in diets for giant freshwater prawn has been shown (Nguyen et al., 2019). Their results showed that up to 60% of fishmeal protein in the diet of the prawn can be substituted by SBY.

4.7.2. Animal feed

SBY as a protein supplement has long been incorporated in ruminant diets. It has been demonstrated under *in vitro* conditions that craft beer SBY, which contain antimicrobial α - and β -acids, as a protein supplement can prevent excessive rumen protein degradation by rumen hyper-ammonia producing bacteria (Harlow, Bryant, Cohen, O'connell, & Flythe, 2016). Black soldier fly (*Hermetia illucens*) larvae, a potential protein source for animal feed, can feed on a wide variety of wastes. It has been shown that the larvae of black soldier fly that fed on spent grain-based diet supplemented with SBY exhibited relatively high wet weight with short doubling time compared with larvae fed unsupplemented diet (Chia et al., 2018a). SBY in combination with

brewers' spent grain and cane molasses were used as substrates for black soldier fly larvae production, which were intended to use as an alternative protein source in aquaculture and livestock feed (Chia et al., 2018b). The incorporation of SBY in pig diet resulted in significantly decreased total cholesterol and blood urea nitrogen in pigs (Sreeparvathy & Anuraj, 2018). They also showed a significant enhancement in apparent magnesium (Mg) availability with SBY incorporation.

It has been shown that corn meal in sheep diet can be substituted up to 100% with SBY without negatively affecting feed intake, feeding behavior and digestibility (Oliveira et al., 2016). SBY in combination with other brewery by-products, namely brewer's spent grain with hot sludge and protein sludge from press liquor, were found suitable for the preparation of protein-rich feed for laying hens (Levic, Djuragic, & Sredanovic, 2010). They showed that the formulated feed exerted superior effect on reproductive performance of laying hens compared with soybean meal. SBY can also replace fish meal as a protein source and can be supplemented in pig diets upto 6% without detrimental effects on performance (Kabugo, Mutetikka, Mwesigwa, Beyihayo, & Kugonza, 2014). In another study, Gondwe, Mtimuni, and Safalaoh (1999) showed that the substitution of vitamin premix with sun-dried SBY in broiler finisher diets exerted significant positive effects in terms of weight gains and live weights, especially in Indian River chickens, compared with birds of control group. In order to improve immune system, the supplementation of ribonucleic acid (RNA) isolated from SBY into cattle feed and human nutrition has been suggested (Chládek, Přikryl, & Zeman, 2007).

4.8. Source of enzymes

Proteases derived from SBY have been used for the production of sardine protein hydrolysates (Vieira, Pinho, & Ferreira, 2017). They showed that, in viscera hydrolysate resulted from SBY proteases treatment, proteins exhibited improved functional properties, including oilbinding capacity, foaming and emulsifying ability. Proteases extracted from SBY were used to hydrolyze sarcoplasmic proteins of sardine byproducts and the resultant hydrolysates were shown to possess ACEinhibitory activity and antioxidant activity (Vieira & Ferreira, 2017). They concluded that two different agro-industrial by-products can be simultaneously valorized through this approach. Proteases from SBY have also been used for the preparation of sardine protein hydrolysate having anti-inflammatory effects (Vieira, Van Camp, Ferreira, & Grootaert, 2018). Their results suggested that the hydrolysate has the potential to be used as a functional food. SBY proteases were used to hydrolyze brewers' spent grain proteins and the resultant hydrolysates exhibited anti-cytotoxic activity (Vieira, da Silva, Carmo, & Ferreira, 2017).

4.9. Source of nucleic acids

SBY is one of the cheapest sources of nucleic acids. For the preparation of flavor-enhancing 5'-nucleotides, SBY has been shown to be an interesting starting material (Sombutyanuchit, Suphantharika, & Verduyn, 2001). It is known that the interaction of 5'-nucleotides synergistically with amino acids (especially glutamic acid) and peptides in yeast extract leads to taste improvement. It has been shown that, for the purpose of intumescent flame retardant coating on cotton fabrics, nucleic acids extracted from SBY can potentially replace expensive, purified low molecular weight DNA (Bosco, Casale, Gribaudo, Mollea, & Malucelli, 2017). Their results concluded that SBY-derived nucleic acids can impart self-extinction to cotton.

4.10. Biosorption

SBY was shown to be an excellent biosorbent for the removal of lead (Pb) from aqueous solution (Duda-Chodak, Tarko, & Milotta, 2012).

Their results showed that SBY can remove > 90% of Pb in the solution (from 200 to 500 mg Pb/dm³ to 1 mg/dm³) in < 20 min. Modified spent yeast was successfully used as a flocculant for harvesting microalgae *Chlorella vulgaris* (Prochazkova, Kastanek, & Branyik, 2015). They showed that highly effective cationic flocculant (> 90% harvesting efficiency) can be developed by surface modification (by treatment with 2-chloro-N, N-diethylethylamine hydrochloride) of microparticles prepared from SBY. Bentonite clay functionalized with spent brewer's yeast was used to recover platinum group metals from wastewater (Mosai, Chimuka, Cukrowska, Kotzé, & Tutu, 2020). Their results suggested that a maximum removal efficiency of 98.5% can be achieved.

5. Miscellaneous applications

SBY could potentially be used as a reducing agent in 'green' synthesis of nanoparticles. SBY has been used for silver nanoparticles (AgNPs) production (Yantcheva et al., 2019). A relatively quick synthesis of AgNPs was observed when non-pasteurized and pasteurized SBY combined with aqueous extract of *Rosa damascena* waste, which is rich in polyphenolic substances.

Carotenoids are widely used as natural dyes in the food industry. SBY along with a by-product of biodiesel production, raw glycerin, was used for microbial production of carotenoids (Rodrigues, Schueler, da Silva, Sérvulo, & Oliveira, 2019). They showed that total carotenoids as high as 420 $\mu\text{g/g}$ can be produced. These results indicate that carotenoids as natural dyes can be produced biotechnologically using SBY as a substrate.

Phytostimulatory effect of SBY has recently been shown. SBY can be used as an elicitor for rye microgreen cultivation (Ana, Andrei, Mircea, Cristina, & Cristina, 2017). They showed that SBY treatment exerted increased germination, fresh mass and plantlet growth. In addition, total phenolic contents and antioxidant activity were enhanced by the treatment.

To decrease atmospheric CO_2 levels, the production and addition of degradation-resistant biochar to soils offers a hopeful way. George, Wagner, Kücke, and Rillig (2012) showed that the addition of hydrochar produced from SBY exhibited positive effects on soil aggregation. However, arbuscular mycorrhizal fungi root colonization was negatively affected by the hydrochar addition.

SBY was used as raw material for the preparation of a protein foaming agent for concrete (Liu, Huo, Lei, & Li, 2010). They showed that foam retaining duration can be extended by the addition of carrageenan to foaming solution.

Different proportions of SBY along with sucrose were supplemented as components in diets used for rearing the Mediterranean fruit fly larvae intended for insect pest control (for the production of sterile male in the sterile insect technique) (Nestel & Nemny-Lavy, 2008).

SBY could be used for the production of small molecules. SBY was successfully used for the co-production of S-adenosyl- ι -methionine and glutathione, with productivities of 40–45 and 13–18 mg/g dry cell weight, respectively (Liu, Lin, Cen, & Pan, 2004).

There has recently been increasing interest in the production of edible insects for human consumption. The potential application of SBY along with other feed ingredients, such as spent grains, cookie remains, wheat bran, soy, rye, oats, etc., for the successful rearing of edible insects has been shown (Halloran, Roos, Eilenberg, Cerutti, & Bruun, 2016).

For cultivating animal cells *in vitro* in basal media supplemented without or with reduced fetal bovine serum, yeast extract derived from SBY along with other inexpensive substrates might be used.

A summary of various valorization opportunities for SBY, which are discussed above, is given in Table 2.

6. Concluding remarks

Economic viability is a key factor for the commercial success of any

Table 2Summary of various valorization opportunities for SBY.

Valorized product/valorization route	Application	
β-Glucans	♦ Emulsion stabilizing agent, water-holding agent, thickener, nutraceutical ingredient, fat replacer	
	◆ Immunostimulant, cryoprotectant for probiotics	
	◆ Triglyceride as well as LDL cholesterol-lowering agent	
	♦ Mycotoxins adsorption	
Proteins and functional peptides	 Antioxidant activity, antiulcer activity, antiproliferative activity, ACE-inhibitory peptides (antihypertensive effect) 	
Yeast extract	♦ Source of protein, fat, trehalose, Bvitamins	
	 ♦ Source of essential amino acids as well as flavor enhancer amino acids (glycine, alanine, glutamic acid and aspartic acid) ♦ Source of macrominerals (K, Na, Ca, Mg) trace elements (Zn, Fe, Mn, Cu, Cr, Co, Mo, Se) and vitamins (B3, B6 and B9) 	
As functional food ingredient Source of bioactive ingredients- phenolic acids, f Improves free amino acid and protein contents Essential amino acids Gel stabilizer, stabilizer in wine Emulsification activity Hop acids (isoα-, α-, and β-acids)	◆ Source of bioactive ingredients- phenolic acids, flavonoids, carotenoids and peptides	
	◆ Improves free amino acid and protein contents	
	◆ Essential amino acids	
	♦ Gel stabilizer, stabilizer in wine	
	◆ Emulsification activity	
	♦ Hop acids (isoα-, α-, and β-acids)	
As fermentation substrate	◆ Proteolytic enzyme or extracellular enzyme production	
	♦ ACE-inhibitory proteins production	
	♦ Succinic acid production	
	◆ Ethanol production	
	♦ Methane (biogas) production	
	♦ L-(+)-Lactic acid production	
As feed ingredient	◆ Fish and prawn feed, cattle feed, poultry feed, swine feed, feed for edible insects	
Miscellaneous	◆ Source of enzymes (e.g. proteases)	
	♦ Source of nucleic acids (e.g. flavor-enhancing 5′-nucleotides)	
	♦ Biosorption (e.g. Pb removal)	
	◆ Flocculant	
	◆ Synthesis of nanoparticles	
	♦ Phytostimulant	
	♦ Bio-oil, activated carbon, hydrochar	

valorization approach. Transportation cost, short shelf life, and tedious processing were reported to be the predominant issues associated with the usage of wet SBY in pig nutrition, especially in developing countries (Boateng et al., 2015). Hence, long-term SBY preservation is an another crucial factor for its effective utilization. The necessity of SBY preservation was highlighted by Bednarski, Leman, and Poznanski (1983) and it was claimed to be a predominant factor preventing widespread utilization of SBY. The addition of either beet molasses (in 1:1 ratio) or urea (3%) to yeast pulp has been suggested for osmoactive preservation of SBY (Bednarski et al., 1983).

Apart from economic viability, sensory attributes of valorized product are crucial if it is intended for use in food products. Bitter flavor of SBY, which arises due to cell wall adsorption of hop-derived resins and tannins during fermentation, may be a hurdle for its inclusion in foods. However, in a previous study, a complete debittering of SBY without affecting chemical composition by adjusting temperature and pH of slurry was reported (Nand, 1987). In another study, rotary microfiltration was used for both debittering as well as debris separation in the process of yeast extract production from SBY (Shotipruk, Kittianong, Suphantharika, & Muangnapoh, 2005). They showed that a considerable reduction in bitterness can be obtained by autolysis of cell homogenate prior to filtration and rotary filtration of the homogenate at pH 5.5.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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